# POSEIDON trial phase 1b results: safety, efficacy and circulating tumor DNA response of the beta isoform-sparing PI3K inhibitor taselisib (GDC-0032) combined with tamoxifen in hormone receptor positive metastatic breast cancer patients.

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**Conflict of interest disclosure statement**

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## STATEMENT OF TRANSLATIONAL RELEVANCE:

The strategy of combining endocrine therapy with PI3K-mTOR inhibition has shown promise in oestrogen receptor-positive breast cancer, but new agents and combinations with a better therapeutic index are urgently needed. Taselisib is a potent, selective, PI3kinase inhibitor. In this phase 1b trial, 30 patients with ER-positive, metastatic breast cancer who had failed prior endocrine therapy were treated with escalating doses of taselisib combined with tamoxifen. The combination was generally well tolerated, with adverse events as expected for this class of drugs, including diarrhea, mucositis and hyperglycemia. No dose-limiting toxicities were observed. Objective responses were seen in 6 out of 25 patients with RECIST-measurable disease (ORR 24%). 12 out of 30 patients (40%) had disease control for 6 months or more. Circulating tumor DNA studies using next-generation tagged amplicon sequencing identified early indications of treatment response and mechanistically-relevant correlates of clinical drug resistance (eg. mutations in *KRAS*, *ERBB2*) in some patients.

**ABSTRACT**:

**Background:** The strategy of combining endocrine therapy with PI3K-mTOR inhibition has shown promise in oestrogen-receptor (ER)-positive breast cancer, but new agents and combinations with a better therapeutic index are urgently needed. Taselisib is a potent, selective, beta-isoform sparing PI3 kinase inhibitor. **Patients and Methods:** 30 patients with ER-positive, metastatic breast cancer who had failed prior endocrine therapy were treated with escalating doses of taselisib (2 or 4 mg in an intermittent or continuous schedule) combined with tamoxifen 20mg once daily in this phase 1b study using a 'rolling six' design. **Results:** Taselisib combined with tamoxifen was generally well tolerated, with treatment-emergent adverse events as expected for this class of drugs, including diarrhea (13 patients, 43%), mucositis (10 patients, 33%) and hyperglycemia (8 patients, 27%). No dose-limiting toxicities were observed. Objective responses were seen in 6 out of 25 patients with RECIST-measurable disease (ORR 24%). Median time to disease progression was 3.7 months. 12 out of 30 patients (40%) had disease control for 6 months or more. Circulating tumor (ct)DNA studies using next-generation tagged amplicon sequencing identified early indications of treatment response and mechanistically-relevant correlates of clinical drug resistance (eg. mutations in *KRAS*, *ERBB2*) in some patients. **Conclusions:** Taselisib can be safely combined with tamoxifen at the recommended phase 2 dose of 4mg given once daily on a continuous schedule. Preliminary evidence of anti-tumor activity was seen in both *PIK3CA* mutant and wild-type cancers. The randomized phase 2 part of POSEIDON (testing tamoxifen plus taselisib or placebo) is currently recruiting.

## INTRODUCTION

The strategy of combining endocrine therapy with inhibitors of the phosphatidylinositol 3–kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway has shown promise in ER-positive breast cancer(1,2), where there is a high prevalence of pathway alterations. However the modest improvement in treatment efficacy when adding these agents has frequently been offset by significant increased toxicity(3).

Taselisib (GDC-0032) is an oral, potent, isoform-selective inhibitor of PI3K alpha, delta and gamma isoforms, with 30-fold less inhibition of PI3K beta relative to alpha (Ki = 0.29nM)(4). In taselisib early clinical development, anti-tumor activity was observed in patients with ER-positive breast cancer, with proportionately more responses in *PIK3CA*-mutant compared with *PIK3CA* wild-type tumors, consistent with preclinical data(5). This was true both for taselisib as a single agent, and also for taselisib in combination with other anti-oestrogens fulvestrant and letrozole(6,7).

Tamoxifen is well established endocrine therapy frequently used for the treatment of ER-positive breast cancer, increasingly in patients who have failed prior endocrine therapies including aromatase inhibitors and/or fulvestrant. To overcome endocrine resistance, CDK4/6 inhibitors have shown to be of added value(8), and are increasingly being utilized in the first or second-line setting. However not all patients derive benefit from a combination with CDK4/6 inhibitors. Inhibition of the PI3K pathway in combination with tamoxifen may be beneficial for a significant proportion of ER-positive patients, most likely in the second- or third-line setting.

We undertook a phase 1b trial to establish the safety, tolerability, and recommended phase 2 dose (RP2D) of taselisib in combination with tamoxifen, for patients with hormone receptor (HR)-positive metastatic breast cancer with progression after prior endocrine therapy. Secondary and exploratory objectives included assessment of pharmacokinetics (PK) and (preliminary) anti-tumor efficacy. Correlative translational studies were performed to identify biomarkers with potential clinical utility, including intensive plasma sampling for circulating tumor (ct)DNA analysis using next generation tagged amplicon sequencing. ctDNA monitoring in early phase clinical trials may have value in drug development(9) for the assessment of biomarkers which can: predict response to therapy(10); provide an early indication of treatment response(11); and shed light on potential mechanisms of acquired drug resistance(12).

## PATIENTS AND METHODS

### Patients

This phase 1b, multi-centre, dose-escalation study was conducted in Amsterdam, Barcelona and Cambridge, UK. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and was approved by regulatory authorities, ethics committees and institutional review boards at each site. All patients had HR-positive breast cancer and provided written informed consent before taking part. Other key inclusion criteria: measurable or non-measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1; age $\geq$ 18 years; life expectancy $\geq$ 12 weeks; fasting glucose $\leq$ 120mg/dL and HbA1c below the upper limit of normal (ULN). Key exclusion criteria: more than 5 prior chemotherapeutic regimens for metastatic breast cancer; presence of untreated, symptomatic or progressive brain metastases; diabetes

158 mellitus requiring anti-hyperglycemic medication; history of thrombo-embolic or
159 inflammatory bowel disease.
160
161
162 **Study Design and Drug Administration**
163 The phase 1b part of the POSEIDON trial reported here used a rolling 6 design to test 3
164 doses/schedules of taselisib tablets in combination with 20mg tamoxifen daily (QD).
165 Cohort 1 tested tamoxifen plus 2mg taselisib QD in a 21 day on / 7 day off intermittent
166 schedule; Cohort 2 tested tamoxifen plus 4mg taselisib QD in a 21 day on / 7 day off
167 intermittent schedule; and Cohort 3 tested tamoxifen plus 4mg taselisib QD in a 28 day
168 continuous schedule. Planned cohort expansions were undertaken in cohorts 2 and 3 to
169 gain additional preliminary data regarding safety, tolerability and efficacy. On cycle 1 day 1,
170 only taselisib was administered for single agent PK studies. Tamoxifen was administered
171 in combination with taselisib from cycle 1 day 2 onwards.
172
173 **Safety & Dose Intensity**
174 Data on Adverse Events (AEs) was collected according to the NCI Common Terminology
175 Criteria for Adverse Events (CTCAE) version 4.03. All AEs were collected regardless of
176 causality until 30-days after the last study drug administration. Dose-Limiting Toxicities
177 (DLTs) were those treatment-emergent AEs occurring during cycle 1 (days 1-28) which
178 warranted a dose-reduction or which were ≥ grade 3 with exceptions listed in
179 Supplementary Methods [SM]. Relative dose intensity of both taselisib and tamoxifen was
180 defined as the actual received dose intensity divided by the intended dose intensity.
181
182 **Plasma Pharmacokinetic and Circulating Tumor (ct)DNA Studies**

Details of plasma taselisib(13) and tamoxifen(14) pharmacokinetic assays, and ctDNA assays(11,12,15) are provided in [SM].

### Tumor Response

Tumor response to treatment was evaluated clinically and also by CT scan assessments every 8 weeks (2 cycles of treatment), with confirmation of objective responses performed ≥ 4 weeks later. Time to progression (TTP) was calculated from start of treatment until progressive disease. All patients had progressed at the time of analysis and therefore no censoring was necessary.

## RESULTS

### Baseline Patient Demographics and Disease Characteristics

From November 2014 to January 2016, 30 patients were enrolled. The cut-off for data analysis was 8 February 2018. Median treatment duration was 4 months (range 1-17). Patients had a median of 2 lines of prior endocrine therapy (range 0-3) and 2 lines of prior cytotoxic chemotherapy (range 0-7) for metastatic disease. Overall 25 out of 30 patients (83%) had received a prior aromatase inhibitor for the treatment of metastatic disease, and 6/30 (20%) prior fulvestrant (Table 1).

### Safety and Tolerability

No DLTs were observed. However, shortly after finishing the DLT window, one patient in cohort 1 developed diarrhea grade 3 due to colitis, therefore the cohort was expanded. As predefined, cohorts 2 and 3 were expanded to confirm safety of these dose levels.

Following independent data monitoring committee review, the RP2D of taselisib in combination with tamoxifen was set at 4mg in a continuous schedule.

The most common treatment-emergent AEs of any grade were elevated liver enzymes (13 out of 30 patients [43%]), diarrhea (43%), anemia (40%) and oral mucositis (33%, Table 2). The majority of these AEs first occurred during the DLT window, persisted during study treatment, but reversed after treatment discontinuation. AEs of special interest occurred in 6 patients (20%): 3 patients had diarrhea grade 3 due to colitis, 2 patients had rash grade 3 and 1 patient developed pneumonitis grade 4. After withholding the study drugs, and treatment with high dose corticosteroids, all recovered to ≤ grade 1.

**Pharmacokinetics**

The concentration-time curves for taselisib in combination with tamoxifen at cycle 1 day 15 are shown in [S1]. Samples from POSEIDON trial are displayed as individual data points against the backdrop of a population PK model from the broader taselisib clinical development programme provided by Genentech. At the taselisib 4mg daily dose level, combining patients on intermittent and continuous schedules, the cycle 1 day 15 median $C_{max}$ for taselisib in combination with tamoxifen was 68.7 ng/mL and median AUC 1070 ng.h/mL, compared with an expected median $C_{max}$ of 59.2 ng/mL (range 33.6-111) and median AUC 1190 ng.h/mL (range 630-2273) from the single agent taselisib population PK model. Endoxifen levels are shown in [S2].

**Anti-tumor activity and *PIK3CA* mutational status**

Six patients had a confirmed RECIST partial response, yielding an objective response rate (ORR) of 24% in the RECIST-measurable group (n=25), or 20% in the overall intention-to-treat population (n=30). Best responses according are shown as a waterfall plot in Figure 1,

alongside an oncoprint plot showing key gene mutations in baseline plasma or tumor tissue samples. Median TTP for the whole population was 4 months (inter-quartile range 2-8), and 8 months for patients achieving a RECIST partial response. The timecourse of responses to treatment are also visualised on a spider plot (Figure 2) and a swimmers plot [S3]. 12 out of 30 patients had disease control for 6 months or more, thus a 6-month clinical benefit rate (CBR) of 40%.

*PIK3CA* mutation testing was done for all patients on baseline tumor tissue and on plasma ctDNA samples. *PIK3CA* mutations were found in 8/30 (27%) of patients (see Oncoprint Figure 1 and mutation lollipop diagram [S4]). In this group of 8 patients with *PIK3CA* mutant tumors, 3 patients had a PR, and the other 5 stable disease as their best response. There was no statistically significant difference for PIK3CA mutant (exon 9, exon 20 or both) vs. wild-type subgroups for either ORR (38% v. 14%) or TTP (153 v. 113 days, respectively).

**Circulating tumor (ct)DNA correlative studies**

All patients had serial plasma sampling for ctDNA correlative studies. Here we describe four patients in whom ctDNA results illustrate molecular correlates with treatment response (Figure 3). ctDNA was not detected in all patients, and our data set was not powered to detect overall correlations in the whole patient group.

In the first case, the patient had previously received weekly paclitaxel and anastrozole as treatment for her *PIK3CA* mutant breast cancer metastatic to bone, lung, and subcutaneous tissues, and was treated with tamoxifen plus taselisib in the 4mg QD continuous schedule. A rapid fall in plasma ctDNA *PIK3CA*$^{H1047R}$ fraction was observed

just 1 week after starting therapy, 7 weeks before her first scheduled CT scan to assess treatment response.

In the second case, the patient had previously received epirubicin, exemestane and capecitabine as treatment for her *PIK3CA* mutant breast cancer metastatic to liver and bone and was treated with tamoxifen plus taselisib in the 4mg QD continuous schedule. She did not respond to treatment and an increase in plasma ctDNA *PIK3CA*$^{H1047R}$ fraction was seen on cycle 1 day 15, six weeks before her end of cycle 2 restaging CT scan.

In the third case, the patient had previously received paclitaxel, anastrozole, everolimus-exemestane, capecitabine, vinorelbine-docetaxel and letrozole to treat her *PIK3CA* wild-type breast cancer metastatic to liver and bones and was treated in the tamoxifen plus taselisib 4mg QD 21/7 intermittent cohort. She did not respond to treatment and increases in plasma ctDNA levels were found for *GATA3* and *KRAS* mutations two weeks ahead of cycle 2 CT scan.

In the fourth case, the patient had previously received paclitaxel, letrozole, docetaxel, capecitabine, exemestane and eribulin to treat her *PIK3CA* wild-type breast cancer metastatic to liver and bones and was treated in the tamoxifen plus taselisib 4mg QD continuous cohort. She did not respond to treatment and increases in plasma ctDNA levels were found for *ERBB2* and *CDH1* mutations 34 and 27 days respectively before she came off trial with disease progression.

## DISCUSSION

Taselisib in combination with tamoxifen is generally well tolerated, with a side effect profile that was manageable, and consistent with taselisib given as a single agent and in

combination with other endocrine agents. In keeping with other PI3K inhibitors, the commonest side effects were diarrhea, anemia, nausea, mucositis and hyperglycemia. Three out of 30 patients had grade 3 colitis, one patient was found to have grade 4 pneumonitis, all of which were reversible. The RP2D of taselisib in combination with tamoxifen was determined to be 4mg on a daily continuous schedule.

Tamoxifen is a pro-drug that is converted to its active metabolites by cytochrome (CYP) P450 enzymes including CYP2D6, CYP3A4, CYP2B6, and CYP2C19. Taselisib is a weak inhibitor of CYP3A4 and does not inhibit any other CYPs in vitro, and did not alter the PK of midazolam, a CYP3A4 substrate, in the first-in-man study of taselisib (PMT4979g). Therefore, no change in taselisib PK was expected when given in combination with tamoxifen. Indeed, the observed taselisib concentrations at day 15 of cycle 1 were in the same range as those of a previously treated single agent taselisib cohort. Also, cycle 2 day 1 Z-endoxifen levels were on average above the laboratory threshold of 5.9 ng/mL(16) in all dose levels.

Preliminary evidence of anti-tumor activity was observed, with confirmed partial responses seen in 6/25 patients with RECIST-measurable disease (ORR 24%). Responses were seen in patients with *PIK3CA*$^{H1047R}$ mutant, *PIK3CA*$^{E545K}$ mutant and *PIK3CA*$^{WT}$ tumors.

A strong rationale exists to explore the combination of PI3K inhibitors with endocrine therapy for the treatment of ER+ breast cancer. The combination of tamoxifen with everolimus improved the median time to progression from 4.5 to 8.6 months in the TAMRAD trial (1), and it is an important research question to test whether or not isoform-selective PI3K inhibitors have therapeutic advantages over TORC inhibitors in this setting. In addition to the POSEIDON trial combination with tamoxifen, taselisib is given in clinical

trials together with fulvestrant (NCT02340221)(17) and letrozole (NCT02273973)(7). Although *PIK3CA* mutations have been implicated in primary endocrine resistance and their prevalence is relatively high (20-25% in ductal breast cancer and 40% in lobular breast cancer), results are conflicting(18,19) and the outcome might depend on the specific mutation that is studied(20).

In the SANDPIPER randomised phase 3 trial (NCT02340221)(17), patients with or without a *PIK3CA* mutation were randomised between taselisib plus fulvestrant and placebo plus fulvestrant. Taselisib dose and schedule were the same as recommended for phase 2 of the POSEIDON study (ie. taselisib 4mg daily continuous). Median PFS with taselisib plus fulvestrant in patients with a *PIK3CA* mutation was significantly longer (7.4 months) than with placebo plus fulvestrant (5.4 months; HR 0.70). No significant PFS difference was observed in patients who had a *PIK3CA* wildtype tumor (median PFS 5.6 months vs 4.0 months). However, information about a test for interaction is lacking. Adverse events grade 3 or higher were observed in almost half of the patients. The toxicity profile seen in POSEIDON is consistent to that reported in previous trials testing taselisib plus endocrine therapy in the metastatic setting. Additionally, in the SOLAR-1 randomised phase 3 trial (NCT02437318)(21) patients with HR-positive, HER2-negative advanced breast cancer were randomised to receive fulvestrant plus the alpha-isoform-selective PI3K inhibitor alpelisib, or placebo. The addition of alpelisib to fulvestrant improved PFS in patients with PIK3CA mutations but not PIK3CA wild-type patients (median PFS 20 vs. 11 months).

Despite these encouraging results, *PIK3CA* mutational status may not on its own be sufficient to optimally identify which ER-positive breast cancer patients will benefit most from the addition of a PI3K inhibitor to endocrine therapy. Individual patients with *PIK3CA* wild-type tumors can respond, and some patients with *PIK3CA* mutant tumors do not.

Further studies are required to identify the optimal biomarker profile for PI3K combination therapy, and how best to use the results of real-time plasma ctDNA monitoring for the management of individual patients. These questions are being addressed in the randomised phase 2 part of POSEIDON which is ongoing.

To conclude, the RP2D of taselisib in combination with tamoxifen 20mg daily is 4mg QD in a continuous schedule. Phase 2 of POSEIDON (NCT02301988) is currently recruiting and randomises patients (*N*=280 in total) to receive tamoxifen 20mg daily with either taselisib 4mg or placebo once daily; including a specific focus on patients with lobular breast cancer (*N*=110); and a major translational effort to identify predictive biomarkers to help select which patients are most likely to benefit from the addition of a PI3K inhibitor to their endocrine therapy.

## REFERENCES

1. Bachelot TD, Bourgier C, Cropet C, Ray-Coquard I, Ferrero J-M, Freyer G, et al. Randomized phase II trial of everolimus in combination with tamoxifen in patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer with prior exposure to aromatase inhibitors: a GINECO study. J Clin Oncol [Internet]. 2012 [cited 2014 Mar 6];30:2718–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22565002
2. Baselga JM, Campone M, Piccart-Gebhart MJ, Burris III HA, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med [Internet]. 2012 [cited 2012 Nov 9];366:520–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22149876
3. Chia S, Gandhi S, Joy AA, Edwards S, Gorr M, Hopkins S, et al. Novel agents and associated toxicities of inhibitors of the PI3k/Akt/mtor pathway for the treatment of breast cancer. Curr Oncol. 2015;22:33–48.
4. Ndubaku CO, Heffron TP, Staben ST, Baumgardner M, Blaquiere N, Bradley E, et al. Discovery of GDC-0032: a β-sparing phosphoinositide 3-kinase inhibitor with high unbound exposure and robust i. J Med Chem [Internet]. 2013 [cited 2014 Mar 14];56:4597–610. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23662903
5. Edgar KA. The PI3K inhibitor, taselisib (GDC-0032), has enhanced potency in PIK3CA mutant models through a unique mechanism of action . AACR Annu Meet. 2016;Abstract no. 370.
6. Juric D, Krop I, Ramanathan RK, Wilson TR, Ware JA, Sanabria Bohorquez SM, et al. Phase I Dose-Escalation Study of Taselisib, an Oral PI3K Inhibitor, in Patients with Advanced Solid Tumors. Cancer Discov [Internet]. 2017;7:704–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28331003
7. Saura C. PD5-2 Ph1b study of the PI3K inhibitor taselisib (GDC-0032) in

combination with letrozole in patients with hormone receptor-positive advanced breast cancer. San Antonio Breast Cancer Symp [Internet]. 2014; Available from: http://www.sabcs.org/Resources/

8. Messina C, Cattrini C, Buzzatti G, Cerbone L, Zanardi E, Messina M, et al. CDK4/6 inhibitors in advanced hormone receptor-positive/HER2-negative breast cancer: a systematic review and meta-analysis of randomized trials. Breast Cancer Res Treat [Internet]. 2018; Available from: http://link.springer.com/10.1007/s10549-018-4901-0

9. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer [Internet]. 2017;17:223–38. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28233803

10. Di Leo A, Johnston S, Lee KS, Ciruelos E, Lønning PE, Janni W, et al. Buparlisib plus fulvestrant in postmenopausal women with hormone-receptor-positive, HER2-negative, advanced breast cancer progressing on or after mTOR inhibition (BELLE-3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol [Internet]. 2018 [cited 2017 Dec 14];19:87–100. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1470204517306885

11. Dawson S-J, Tsui DWY, Murtaza M, Biggs H, Rueda OM, Chin S-F, et al. Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer. N Engl J Med [Internet]. 2013 [cited 2013 Mar 15];368:1199–209. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa1213261

12. Murtaza M, Dawson S-J, Tsui DWY, Gale D, Forshew T, Piskorz AM, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature [Internet]. Nature Publishing Group; 2013 [cited 2013 May 22];497:108–12. Available from: http://www.nature.com/doifinder/10.1038/nature12065

13. Ding X, Faber K, Shi Y, McKnight J, Dorshorst D, Ware JA, et al. Validation and determination of taselisib, a β-sparing phosphoinositide 3-kinase (PI3K) inhibitor, in human plasma by LC-MS/MS. J Pharm Biomed Anal [Internet]. Elsevier B.V.; 2016;126:117–23. Available from: http://dx.doi.org/10.1016/j.jpba.2016.04.030

14. Teunissen SF, Jager NGL, Rosing H, Schinkel AH, Schellens JHM, Beijnen JH. Development and validation of a quantitative assay for the determination of tamoxifen and its five main phase I metabolites in human serum using liquid chromatography coupled with tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci [Internet]. 2011;879:1677–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21543272

15. Gao M, Callari M, Beddowes E, Sammut S-J, Grzelak M, Biggs H, et al. Next Generation-Targeted Amplicon Sequencing (NG-TAS): An optimised protocol and computational pipeline for cost-effective profiling of circulating tumour DNA. bioRxiv [Internet]. 2018; Available from: https://www.biorxiv.org/content/early/2018/07/15/366534

16. Madlensky L, Natarajan L, Tchu S, Pu M, Mortimer J, Flatt SW, et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clin Pharmacol Ther. 2011;

17. Baselga JM. Phase III study of taselisib (GDC-0032) + fulvestrant (FULV) v FULV in patients (pts) with estrogen receptor (ER)-positive, PIK3CA-mutant (MUT), locally advanced or metastatic breast cancer (MBC): Primary analysis from SANDPIPER. ASCO Annu Meet [Internet]. 2018;LBA1006. Available from: https://meetinglibrary.asco.org/browse-meetings/2018 ASCO Annual Meeting

18. Beelen K, Opdam M, Severson TM, Koornstra RHT, Vincent AD, Wesseling J, et al. PIK3CA mutations, phosphatase and tensin homolog, human epidermal growth factor receptor 2, and insulin-like growth factor 1 receptor and adjuvant tamoxifen

resistance in postmenopausal breast cancer patients. Breast Cancer Res [Internet]. 2014;16:R13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24467828

19. Beelen K, Zwart W, Linn SC. Can predictive biomarkers in breast cancer guide adjuvant endocrine therapy? Nat Rev Clin Oncol [Internet]. Nature Publishing Group; 2012 [cited 2012 Oct 22];9:529–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22825374

20. Ellis MJ, Lin L, Crowder R, Tao Y, Hoog J, Snider J, et al. Phosphatidyl-inositol-3-kinase alpha catalytic subunit mutation and response to neoadjuvant endocrine therapy for estrogen receptor positive breast cancer. Breast Cancer Res Treat [Internet]. 2010 [cited 2011 Oct 4];119:379–90. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2810126&tool=pmcentrez&rendertype=abstract

21. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med 2019: 380:1929-40

444 # TABLE & FIGURE HEADERS

445

446 **Table 1. Patient baseline characteristics.**

447

448 **Table 2. Most frequently observed treatment-emergent adverse events.**

449

450 **Figure 1. Anti-tumor activity and pre-treatment tumor genetics (all patients, N=30).**

451 a) Waterfall plot showing best treatment response for all 30 patients – 25 with RECIST-
452 measurable disease and 5 with non-measurable disease (the latter marked by an
453 asterisk*). Best RECIST response and time on treatment in months are indicated for each
454 patient. PR - partial response, SD - stable disease, PD - progressive disease).
455 b) Oncoprint plot showing pre-treatment mutation status of PIK3CA, PIK3R, PTEN,
456 MAP3K1 and TP53 genes. In each square, detection of a mutation in the tissue (primary or
457 metastatic) is shown on the left side, while detection on plasma (at baseline) is shown on
458 the right. Cases where tissue was not available are indicated in dark grey; for all the others,
459 both tissue and plasma were tested. The black outline indicates that the mutation is
460 present in Cosmic database. The white star indicates mutations in tissue and plasma are
461 not in the same position. Numbers on the top indicate the exon of PIK3CA mutations (9 or
462 20); T–tumor, P–plasma.

463

464 **Figure 2. Spider plot showing change in tumor size over time for individual patients**
465 **with RECIST-measurable disease (*N*=25).**

466

467
468 **Figure 3. Circulating tumor (ct)DNA correlative case studies.**

469 In four individual patients each having different clinical outcomes, the variant allele fraction
470 is shown over time for gene mutations in plasma whilst on study treatment.

**S1. Supplementary Figure 1. Taselisib pharmacokinetics in combination with tamoxifen.**

**S2. Supplementary Figure 2. Pharmacokinetics: Z-endoxifen levels per taselisib dose level.**

**S3. Supplementary Figure 3. Time to progression per patient in a swimmers plot (all patients, N=30).**

**S4. Supplementary Figure 4. PIK3CA mutations detected at baseline (in samples from 8 out of 30 patients).**

| | Cohort 1 (*N*=6)<br>2mg Taselisib QD 21d, 7d off<br>+ 20mg tamoxifen | Cohort 2 (*N*=13)<br>4mg Taselisib QD 21d, 7d off<br>+ 20mg tamoxifen | Cohort 3 (*N*=11)<br>4mg Taselisib continuous<br>+ 20mg tamoxifen | All patients<br>(*N*=30) |
|---|---|---|---|---|
| **Age in years – median (range)** | 51 (41–68) | 54 (45–72) | 54 (35–81) | 53 (35–81) |
| **ECOG Performance Status** | | | | |
| **0** | 3 (50%) | 3 (23%) | 5 (45%) | 11 (37%) |
| **1** | 3 (50%) | 10 (77%) | 6 (55%) | 19 (63%) |
| **Histological subtype** | | | | |
| **Ductal** | 4 (67%) | 12 (92%) | 8 (73%) | 24 (80%) |
| **Lobular** | 2 (33%) | 1 (8%) | 2 (18%) | 5 (17%) |
| **Unknown** | 0 | 0 | 1 (9%) | 1 (3%) |
| ***PIK3CA* mutational status** | | | | |
| **Wild type** | 5 (83%) | 13 (100%) | 8 (73%) | 26 (87%) |
| **H1047R mutation** | 1 (17%) | 0 | 2 (18%) | 3 (10%) |
| **E545K mutation** | 0 | 0 | 1 (9%) | 1 (3%) |
| **Number of prior metastatic therapies – median (range)** | 2 (1-2) | 2 (1-3) | 2 (0-3) | 2 (0-3) |
| **Endocrine** | 2 (0-7*) | 1 (0-5) | 2 (0-5) | 2 (0-7*) |
| **Cytotoxic** | | | | |
| **Prior endocrine therapies for metastatic disease** | | | | |
| **Tamoxifen** | 1 (17%) | 4 (31%) | 1 (9%) | 6 (20%) |
| **Aromatase inhibitor** | | | | |
| - **Anastrozole** | 0 | 3 (23%) | 4 (36%) | 7 (23%) |
| - **Letrozole** | 2 (33%) | 6 (46%) | 4 (36%) | 12 (40%) |
| - **Exemestane** | 3 (50%) | 4 (31%) | 2 (18%) | 8 (27%) |
| **Fulvestrant** | 2 (33%) | 2 (15%) | 2 (18%) | 6 (20%) |
| **Megestrol acetate** | 0 | 1 (8%) | 0 | 1 (3%) |

* after data cleaning it was found that 1 patient had received more than 5 prior lines of cytotoxic chemotherapy

**Table 1. Patient Baseline Characteristics**

| | Cohort 1 (*N*=6) 2mg Taselisib QD 21d, 7d off + 20mg tamoxifen | | Cohort 2 (*N*=13) 4mg Taselisib QD 21d, 7d off + 20mg tamoxifen | | Cohort 3 (*N*=11) 4mg Taselisib continuous + 20mg tamoxifen | | All patients (*N*=30) | |
|---|---|---|---|---|---|---|---|---|
| | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 | Grade 1-2 | Grade 3-4 |
| **AST/ALT/GGT increase** | 1 (17%) | 0 | 5 (38%) | 3 (23%) | 4 (36%) | 0 | 10 (33%) | 3 (10%) |
| **Diarrhea/colitis*** | 2 (33%) | 1 (17%)* | 5 (38%) | 1 (8%)* | 3 (27%) | 1 (9%)* | 10 (33%) | 3 (10%) |
| **Anemia** | 3 (50%) | 0 | 3 (23%) | 0 | 6 (54%) | 0 | 12 (40%) | 0 |
| **Mucositis – oral** | 2 (33%) | 0 | 2 (15%) | 2 (15%) | 4 (36%) | 0 | 8 (27%) | 2 (7%) |
| **Nausea** | 2 (33%) | 0 | 5 (38%) | 0 | 2 (18%) | 0 | 9 (30%) | 0 |
| **Hyperglycemia** | 0 | 0 | 3 (23%) | 0 | 4 (36%) | 1 (9%) | 7 (23%) | 1 (3%) |
| **Lipase/amylase increase** | 1 (17%) | 0 | 2 (15%) | 2 (15%) | 1 (9%) | 2 (18%) | 4 (13%) | 4 (13%) |
| **Fatigue** | 0 | 0 | 3 (23%) | 0 | 4 (36%) | 0 | 7 (23%) | 0 |
| **Headache** | 1 (17%) | 0 | 5 (38%) | 0 | 1 (9%) | 0 | 7 (23%) | 0 |
| **Thrombocytopenia** | 1 (17%) | 0 | 3 (23%) | 1 (8%) | 1 (9%) | 0 | 5 (17%) | 1 (9%) |
| **Alopecia** | 0 | 0 | 4 (31%) | 0 | 1 (9%) | 0 | 5 (17%) | 0 |
| **Weight loss** | 1 (17%) | 0 | 1 (8%) | 0 | 3 (27%) | 0 | 5 (17%) | 0 |
| **Abdominal pain** | 1 (17%) | 0 | 1 (8%) | 0 | 2 (18%) | 0 | 4 (13%) | 0 |
| **Creatinine increase** | 0 | 0 | 3 (23%) | 0 | 1 (9%) | 0 | 4 (13%) | 0 |
| **Triglyceride increase** | 0 | 0 | 2 (15%) | 0 | 1 (9%) | 1 (9%) | 3 (10%) | 1 (3%) |
| **Pneumonitis** | 0 | 0 | 0 | 1 (8%) | 0 | 0 | 0 | 1 (3%) |
| **Any AE** | **4 (67%)** | **1 (17%)** | **5 (38%)** | **8 (62%)** | **6 (54%)** | **5 (45%)** | **15 (50%)** | **14 (47%)** |

**Table 2. Most frequently observed treatment-emergent adverse events.**

Highest grade of AEs occurring at any time point, in at least 10% of patients, and thought to be at least possibly study-drug related.
AE – adverse event; AST – aspartate transaminase; ALT – alanine transaminase; GGT – gamma glutamyltransaminase; QD – once daily.

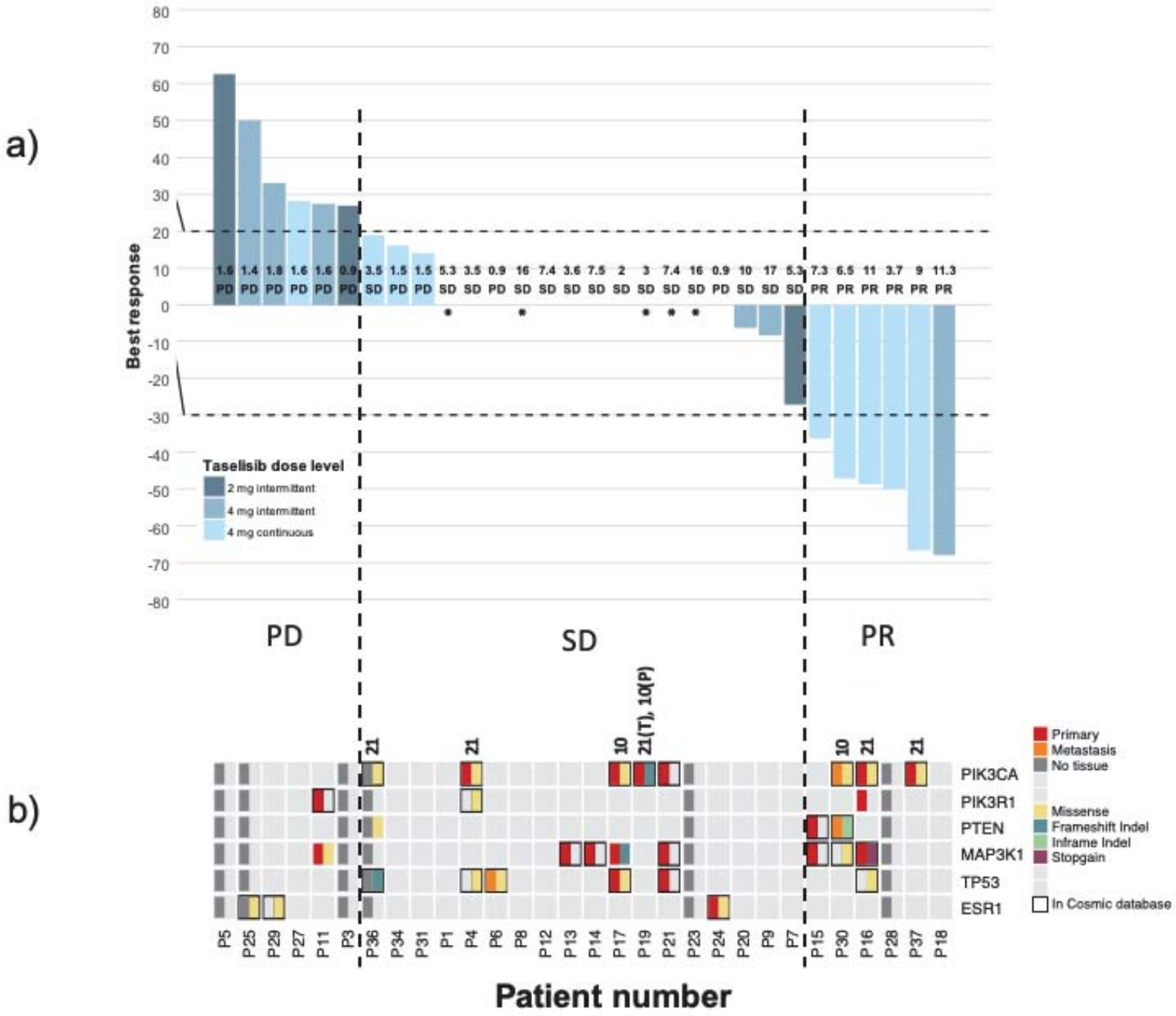



**Figure 1.**

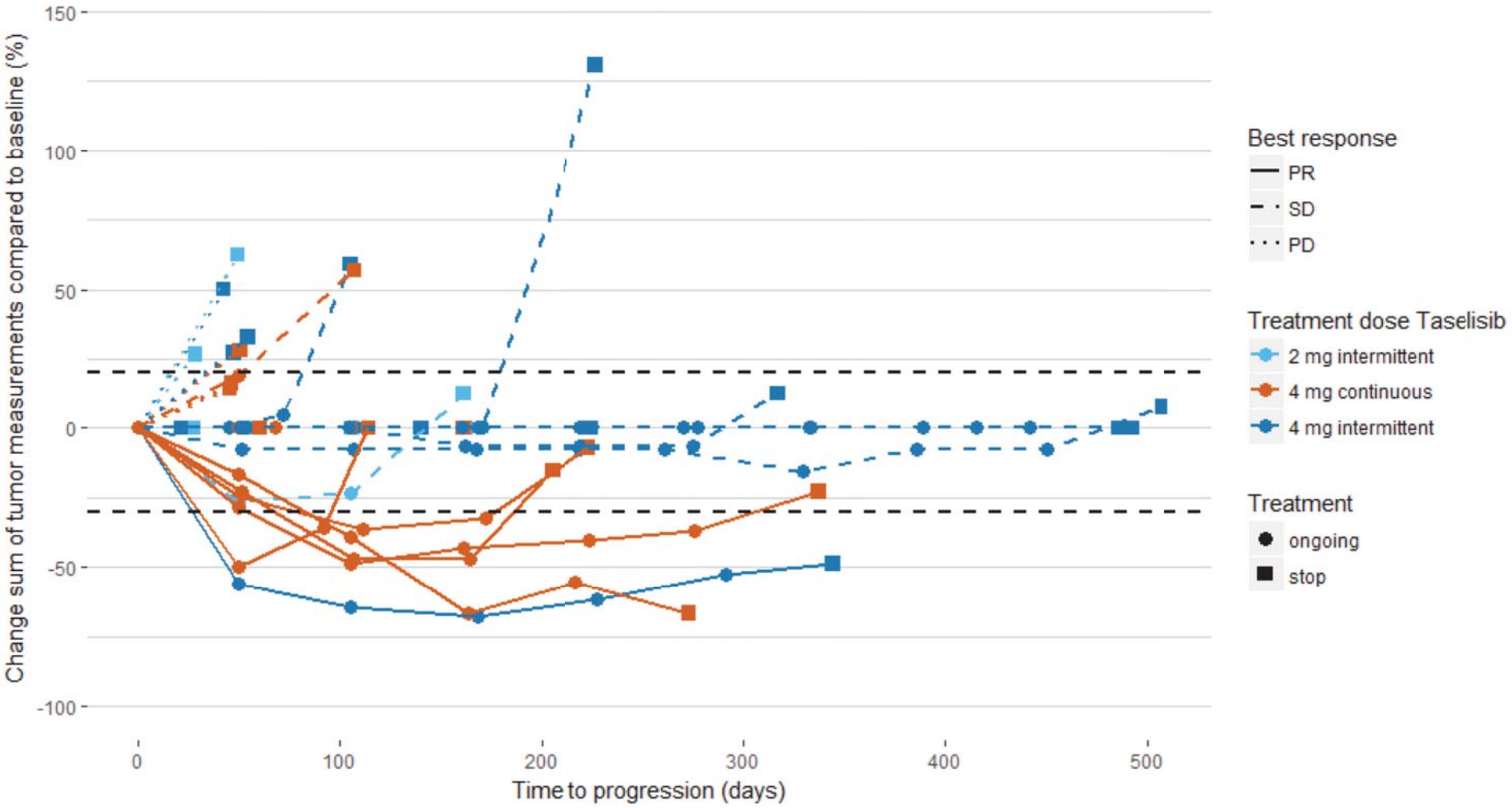
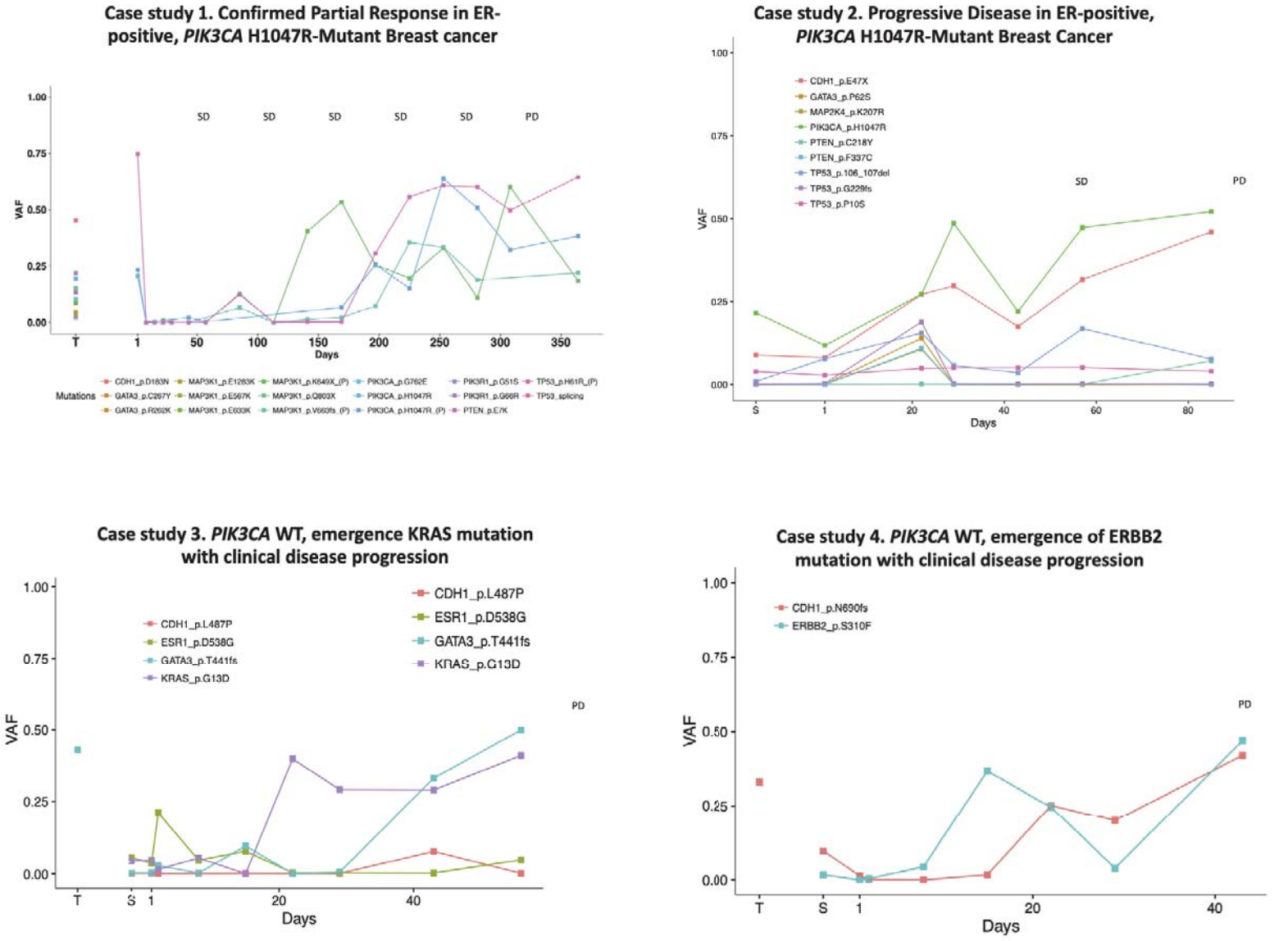



**Figure 2**




CDH1 – cadherin 1; CT – computed tomography; ERBB2 – Erb-B2 Receptor Tyrosine Kinase 2; GATA3 – GATA Binding Protein 3; KRAS – Kirsten ras oncogene homolog; PIK3CA – Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; VAF – variant allele fraction; WT – wild type.
S = screening. T = pre-treatment tumour sample.

**Figure 3.**

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# POSEIDON phase 1b results: safety, efficacy and ctDNA response of taselisib combined with tamoxifen in hormone receptor positive metastatic breast cancer patients

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